user manual
proteomics

Ettan *Spot Picker*

Instrument Handbook

um 18-1147-62

Amersham Biosciences

Important user information

All users must read this entire manual to fully understand the safe use of Ettan™ Spot Picker.

WARNING!

The Warning sign highlights an instruction that must be strictly followed in order to avoid personal injury. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Caution!

The Caution sign is used to call attention to instructions or conditions that must be followed to avoid damage to the product or other equipment. Be sure not to proceed until the instructions are clearly understood and all stated conditions are met.

Declaration of conformity

Safety standards

This product meets the requirements of the Low Voltage Directive 72/23/EEC through the harmonized standard EN 61 010-1:1993 + A2:1995.

EMC standards

This product meets the requirements of the EMC Directive 89/336/EEC through the harmonized standards EN 50081-1 and EN 50082-1.

WARNING!

This is a Class A product. In a domestic environment, this product may cause radio interference, in which case the user may be required to take adequate measures.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences except for alterations described in this manual.

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1 Introduction

The Ettan™ Spot Picker is designed to automatically excise and transfer polyacrylamide gel plugs, containing proteins, from 2D gels to microplates. These gel plugs are suitable for further processing prior to mass spectrometry analysis.

The Spot Picker is compatible with 2D gels that have been post-stained with Coomassie™, silver or SYPRO™ dyes, and for pre-labelled proteins.

Spots of interest can be evaluated and selected by using the ImageMaster™ 2D Elite gel image analysis software. A pair of reference markers must be included within the gel and also detected during image analysis. These reference markers are used to transform image (pixel) co-ordinates into distance values for spot picking.

Following excision from the gel, gel plugs are dispensed into 96-well microplates, along with a volume of dispensing fluid.

1.1 The Ettan Spot Picker system

Fig 1-1. The Ettan Spot Picker system.

The Ettan Spot Picker system comprises the following:

- Ettan Spot Picker instrument
- Ettan Spot Picker instrument control software, running under Windows NT operating system on a PC
- PC with frame grabber card for video display

The package contents are described in Section 1.2. A detailed description of the Ettan Spot Picker instrument is provided in Chapter 7 of this manual.

1.1.1 Rating labels

A rating label is located on the rear panel of the Ettan Spot Picker instrument, see Fig. 1-2. Pay attention to the component ratings. These ratings determine the degree of electrical hazards in the equipment connected to the supply voltage. There are, however, other hazards, which may be more severe, see Chapter 2 Safety instructions.

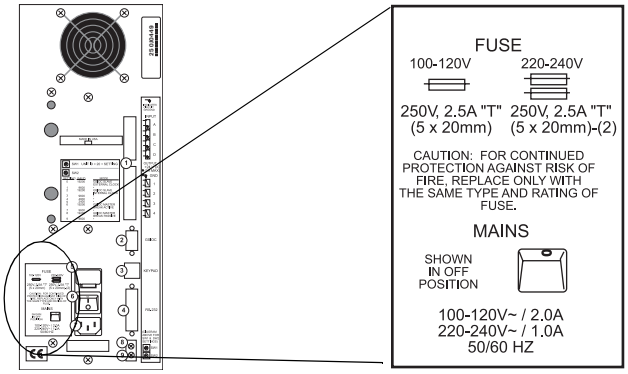


Fig 1-2. Location and layout of rating labels.

1.2 Package contents

The Ettan Spot Picker instrument is delivered with the products listed in Table 1-3 . Picker heads can be ordered separately, see ordering information in Chapter 7.

Product	*Quantity*
Ettan Spot Picker Instrument Control Software	1
Picker head 1.4 mm for 1 mm thick gels	2
Picker head 1.4 mm for 1.5 mm thick gels	2
Reference markers, sheet incl. 560 pieces	2
Gel holder	2
Gel tray	1
Glass lid for gel tray	1
Inlet tubing package	1
Waste bottle and tubing	1
Accessories for camera calibration: camera calibration foot white sheet for camera calibration and click-and-pick function	 5 2
Microplate rack	2
RS 232 cable	1

Table 1-3. Ettan Spot Picker, delivered products.

1.3 Accessories

In addition to the items delivered with the system, Ettan Spot Picker requires the following accessories:

- image scanner: Image Scanner or Typhoon*
- gel image evaluation software, e.g. ImageMaster 2D Elite
- 96-well microplates

See Chapter 7 for specifications and ordering information.

*If you want to use other scanners, please contact Amersham Biosciences.

1.4 The Instrument Handbook

This handbook provides safety instructions, technical information and basic operating instructions for the Ettan Spot Picker instrument. In addition, maintenance schedules and instructions for user maintenance are included.

1.5 Associated documentation

The *Ettan Spot Picker User Manual* describes the experimental use of the instrument, and includes descriptions of all software dialogues.

Gilson™ 215 Liquid Handler User's Guide describes the unmodified instrument and contains technical specifications and instructions for installation and maintenance. Reference will be made to instructions located in the *User's Guide*.

The *Sony™Black-and-White Video Camera Module XC-55/55BB Operating Instructions* describes the camera.

The *Matrox™ Meteor-II Installation and Hardware Reference manual* describes the frame grabber board and provides installation instructions.

2 Safety instructions

IMPORTANT! To avoid any risk of injury, the instrument should only be operated by properly trained personnel and always in accordance with the instructions provided.

The purpose of this chapter is to describe safety precautions and present all safety labels that are attached to the instrument. Built-in safety functions are described, and emergency and disposal procedures are included.

2.1 Safety precautions

1 Read this entire manual before using the Ettan Spot Picker instrument.

2 This instrument is designed for indoor use only.

3 The instrument must always be used with the protective earth lead of the power cord correctly grounded to earth at the mains outlet.

4 To permit sufficient cooling, ensure that the vents at the top and bottom of the instrument are not covered.

5 Do not operate the instrument in extreme humidity (above 95%). Avoid condensation by letting the unit equilibrate to ambient temperature.

6 Do not operate the instrument in direct sunlight.

7 Keep the instrument dry and clean. Wipe regularly with a soft damp tissue. Let the instrument dry completely before use.

8 Any equipment connected to the instrument should meet the requirements of the EN 61 010-1 or other international safety standards.

WARNING! Do not use the Ettan Spot Picker in any other way than described in this Instrument Handbook. The protection provided by the instrument may be weakened if other operations are performed.

2.2 Safety labels

The following safety labels are attached to the Ettan Spot Picker instrument to warn the user of potentially hazardous conditions:

At the picker head

Fig 2-1. Safety label located on the Y-arm, near the picker head.

WARNING! MOVING PARTS. The picker head and camera assembly can make sudden, rapid movements. Keep all body parts clear when the Spot Picker is in operation.

WARNING! Never put your hands underneath the picker head during instrument operation. The picker head has the capacity to puncture skin. In the event of an emergency, press the **Stop** button on the Spot Picker: the instrument immediately ceases movement and all motors are de-energized.

WARNING! To prevent fire or shock hazard, do not spill liquids into the camera body.

2.3 Other warnings

WARNING! HEAVY OBJECT. The Ettan Spot Picker weighs about 40 kg. Two persons are required to lift the instrument.

WARNING! NO SERVICEABLE PARTS INSIDE. Do not open covers. Service and maintenance should be performed by qualified personnel.

2.4 Emergency procedures

2.4.1 Emergency shutdown

In the event of an emergency, press the **Stop** button on the Ettan Spot Picker front panel. The instrument immediately ceases movement and all motors are de-energized.

2.4.2 Power failure routine

In the event of a power failure, the experiment is interrupted in an undefined state. However, the data for gel plugs picked, up to the power interruption, is saved.

When power returns, this will happen:

1 The picker head and the syringe pump (dilutor) are homed.

2 The PC starts and displays the log-in dialogue.

2.4.3 Restart procedure

In the event of system shutdown due to power failure, emergency stop or process interruption, malfunctions must be rectified before the Ettan Spot Picker is restarted.

To restart the Ettan Spot Picker:

1 Check that the **Power** indicator on the Ettan Spot Picker instrument lights.

2 Select **Programs:Ettan Spot Picker:Ettan Spot Picker** from the Windows **Start** menu.

3 Reload the result file and continue picking, see instructions in *Ettan Spot Picker User Manual*, *Chapter 6*, Section *Resume picking after Ettan Spot Picker has been stopped*.

Note: *The last picked gel plug may be lost. This depends on the exact moment when the interruption occurred.*

3 Basic Operation

This chapter provides basic operating instructions for the Ettan Spot Picker instrument, including starting, stopping and preparing for gel loading. Refer to the *Ettan Spot Picker User Manual* for instructions on picking gels.

It is assumed that the instrument and the PC have been correctly installed by Amersham Biosciences personnel.

3.1 Preparing the instrument

CAUTION! Cleanliness is very important for subsequent analysis of the gel plugs. Always wear protective gloves when handling gel and microplates.

WARNING! The picker head and camera assembly can make sudden, rapid movements. Make sure that power to the Spot Picker is disconnected while working with the gel tray, racks and picker head.

3.1.1 Tubing

Plumbing connections:
See *Gilson 215 Liquid Handler User's Guide*, Installation chapter.

Before starting a run, a liquid supply of double-distilled water, or the wash/destain buffer must be connected to the picker head, via the syringe pump. The waste tube from the rinse station should be connected to a waste bottle, placed beneath the instrument (see Fig. 3-1).

The liquid is used for aspirating gel plugs and dispensing them into the wells of the microplates. The picker head is rinsed in liquid at the rinse station between each pick.

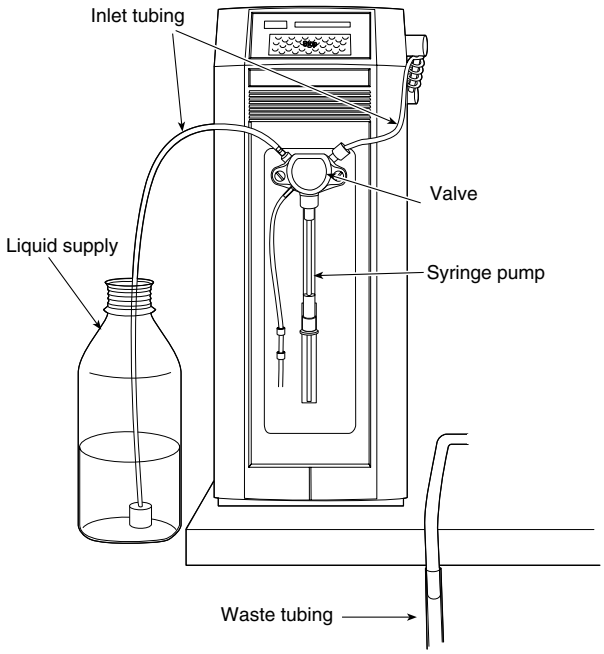


Fig 3-1. Spot Picker tubing setup.

3.1.2 Positioning the gel tray

Place the gel tray on the left-hand part of the locator plate as shown in Fig. 3-2. The guide feet on the tray should fit onto pins on the locator plate. It is only possible to fit the gel tray in one direction, with the corner-mounted guide feet facing the operator (see Fig. 3-3).

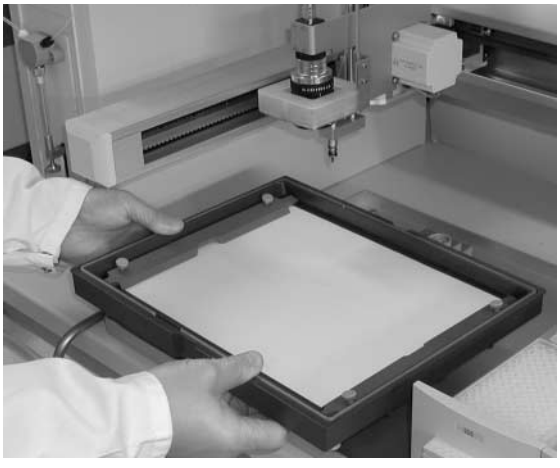


Fig 3-2. Placing the gel tray on the locator plate.

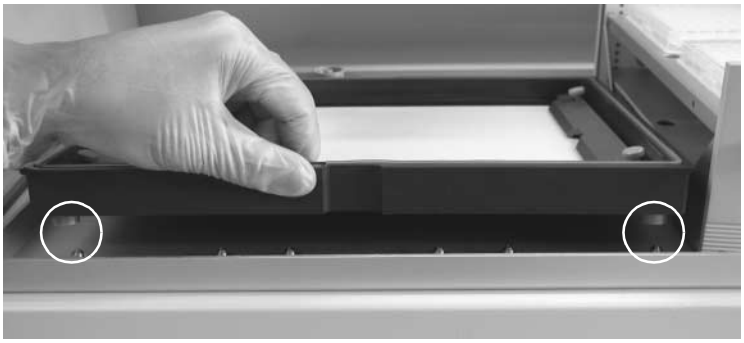


Fig 3-3. Positioning the gel tray.

The gel to be picked is fastened in the gel tray using adjustable clamps (gel holders), as shown in Fig. 3-4.

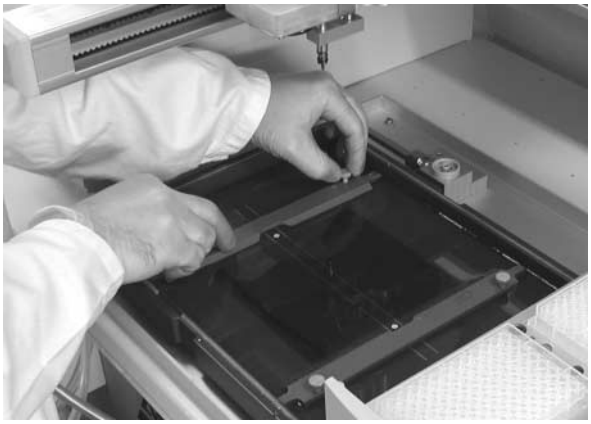


Fig 3-4. Fastening the gel.

3.1.3 Racks and microplates

The gel plugs are collected in microplates, which are placed in two racks on the right-hand side of the locator plate.

1 Install the racks on the locator plate, aligning the holes on the underside with the pins on the locator plate (see Fig. 3-5). The rack label should face the front of the Spot Picker.

2 Place up to four microplates in the racks. Note that well **A1** should face the front of the instrument, see Fig. 3-6.

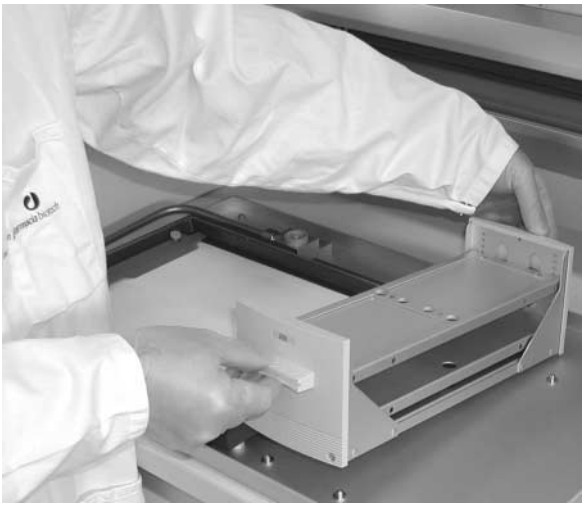


Fig 3-5. Placing a microplate rack on the locator plate.

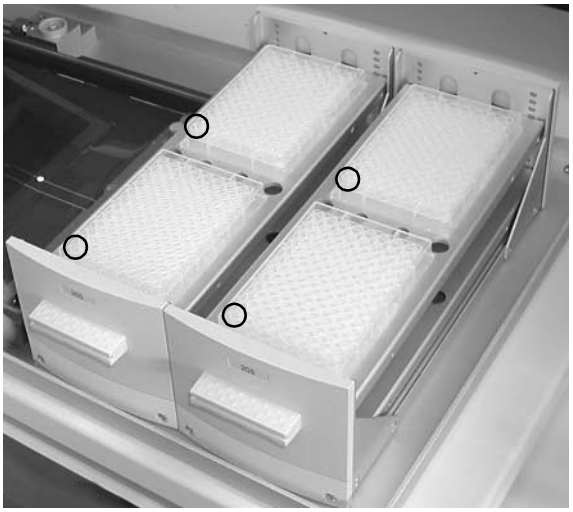


Fig 3-6. Installing microplates in the racks, well A1 marked.

3.2 Selecting a picker head

Picker heads are available in different sizes for different types of gel. See Chapter 7 for specifications. If you need to change the picker head, follow the instructions in Chapter 4, Maintenance. After changing the picker head, a camera calibration should be performed. Instructions for calibration can also be found in Chapter 4.

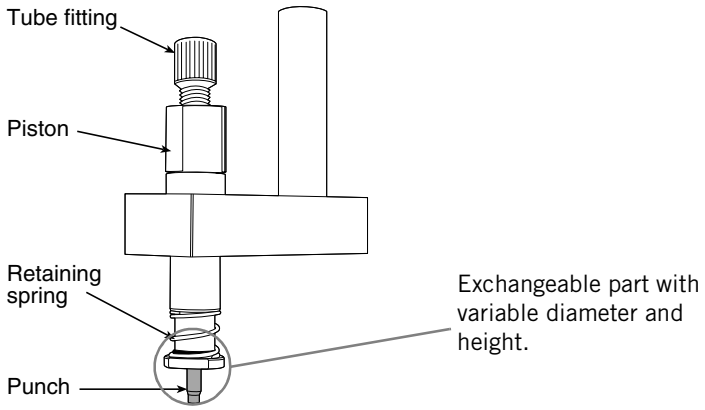


Fig 3-7. The picker head.

3.3 Starting Ettan Spot Picker

WARNING! The picker head and camera assembly can make sudden, rapid movements. Keep all body parts clear when the Spot Picker is in operation.

1 Check that the Spot Picker instrument is connected to the PC (see Chapter 6 Moving and reinstalling the system).

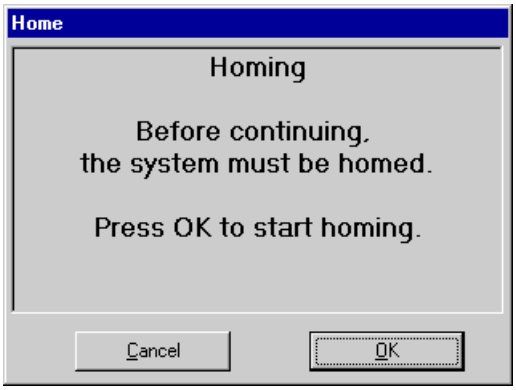
Note: The Spot Picker control software will not run unless a frame grabber card is installed in the computer.

2 Switch on the mains power at the rear of the instrument.

3 Turn on the PC to which it is connected.

4 To start the Ettan Spot Picker Instrument Control Software, select **Programs:Ettan Spot Picker:Ettan Spot Picker** from the Windows **Start** menu:

5 The picker head will be moved to the home position when the software starts. For safety reasons, the user must confirm the movement before starting:



6 After homing, which takes about 20 seconds, the picker head is positioned as shown in Fig. 3-8.

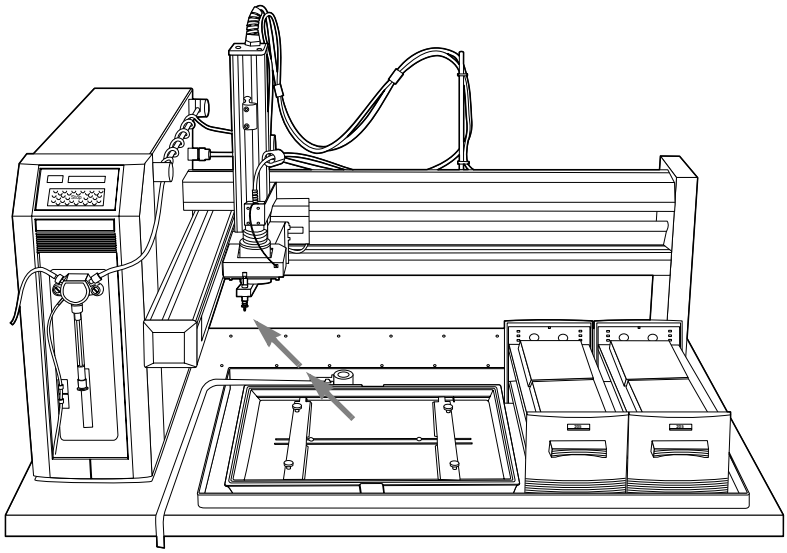


Fig 3-8. Homing the picker head.

3.4 Software user interface

When the homing procedure is ready, the main application window is shown. You can control the Spot Picker by using buttons in the window, and by selecting functions from the pull-down menus at the top of the screen.

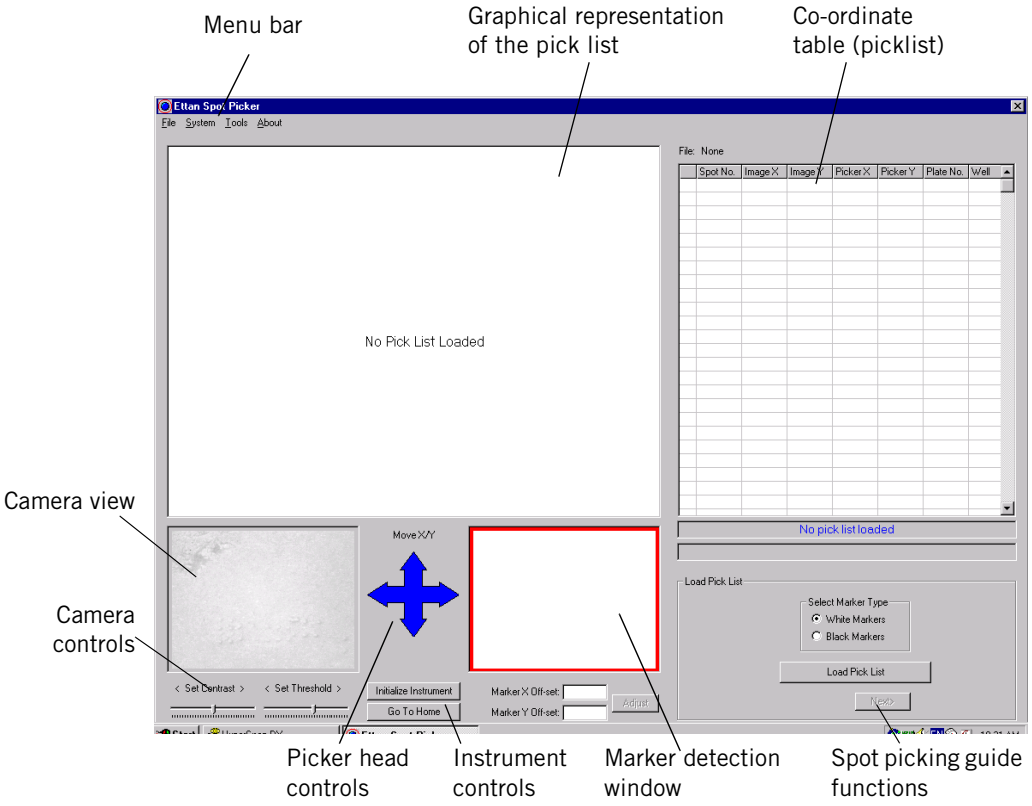


Fig 3-9. The Ettan Spot Picker main application window.

For detailed descriptions of the Ettan Spot Picker software functions, see *Ettan Spot Picker User Manual*.

3.5 Setting system parameters

The following parameters must be set up before starting a picking run:

- The position of the rinse station and the microplates
- The height of the gel backing material (Gel Z-position)
- The parameters used for picking the gel plugs
- The acceptable area range for the reference markers (in pixels)

This section describes the procedures for setting the positions of the rinse station and the microplates. For instructions on setting parameters for gel handling, see *Ettan Spot Picker User Manual*.

To set the Spot Picker parameters choose **System:System Setup.**

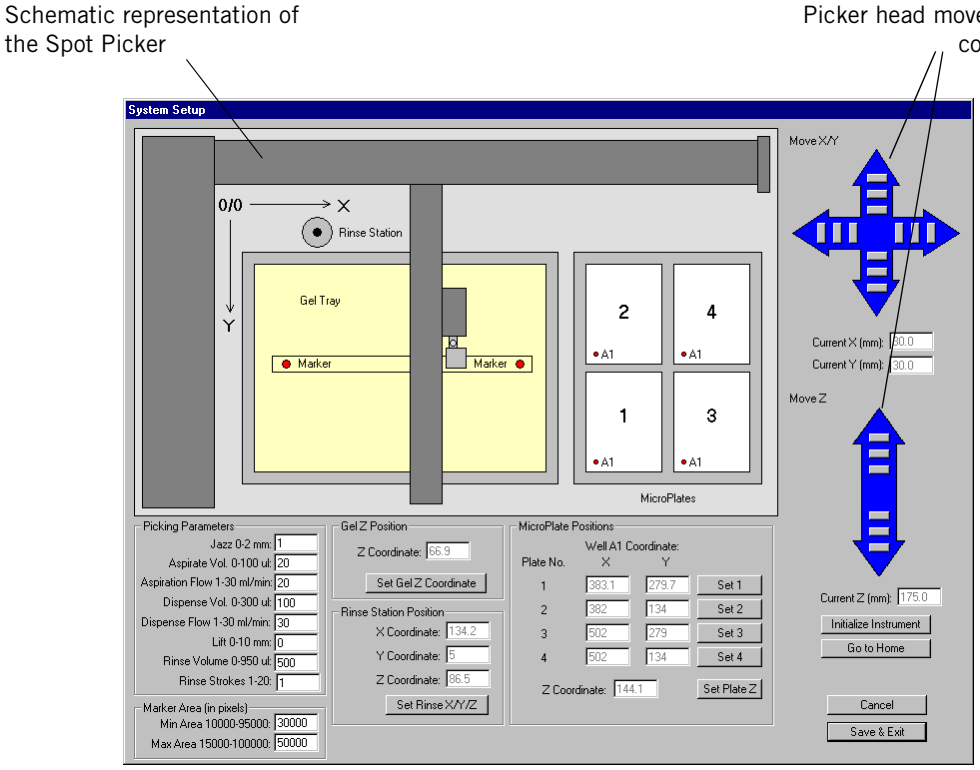
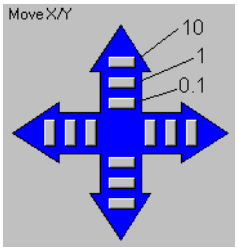


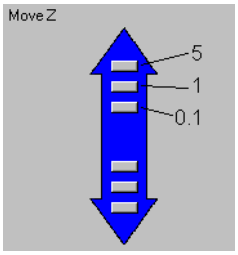
Fig 3-10. The System Setup screen.

3.5.1 Moving the picker head around

It is advisable to press the **Go to home** button prior to setting any of the system locations.



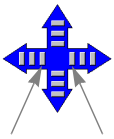
The blue arrows on the right hand side of the screen are used to move the picker head to various positions. By pushing the buttons on the arrows, the head will move stepwise in the corresponding directions. As long as a button is pushed, the head will keep moving:



Move X/Y	move the picker head **horizontally**: the outermost buttons will move the head in 10 mm steps, the middle buttons in 1 mm steps and the innermost buttons in 0.1 mm steps.
Move Z	move the picker head **vertically** in 5, 1 and 0.1 mm steps, respectively.

Note: When the picker head is close to the rinse station, the 10 mm step button can be disabled, since the rinse station is located near the horizontal limit of the Spot Picker. Use the 1 mm and 0.1 mm buttons to move the picker head at this stage.

CAUTION! Be careful when using the **Move Z** buttons; rapidly lowering the picker head into an object may damage the picker head. If the picker head hits an obstacle, the motor will be shut off and an error message will be displayed.



CAUTION! Be careful when using the **Move X/Y** buttons if the picker is in a low Z-position. Rapidly moving the picker head into an object may damage the picker head. Always use the innermost **Move X/Y** buttons when adjusting the sideways position at low Z-heights.

The current X/Y/Z-position of the picker head is continuously read back from the instrument and displayed in the corresponding text boxes.

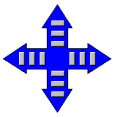
Once the picker head is placed appropriately (e.g. in the rinse station), the location can be stored by pressing the appropriate **Set** button.

If the **Cancel** button is pressed, any changes made to the System Setup will be lost. To retain new System Setup parameters, click on the **Save & Exit** button.

3.5.2 Camera calibration before picking

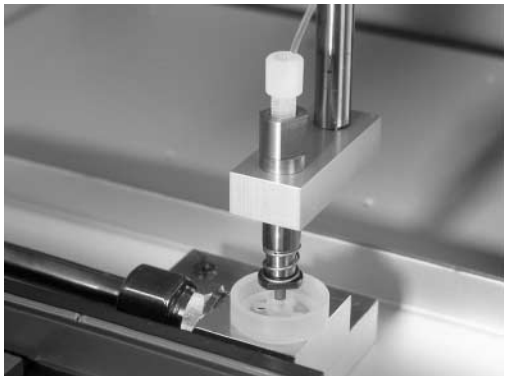
If the picker head has been replaced, the distance between the camera and the picker head needs to be calibrated before starting a picking run. See instructions in Chapter 4.

3.5.3 Setting the position of the rinse station

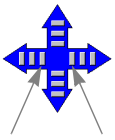


1 Using the **Move X/Y** arrow, move the picker head until it is placed above the centre of the rinse station.

2 Using the **Move Z** arrow, slowly lower the picker head into the rinse station.



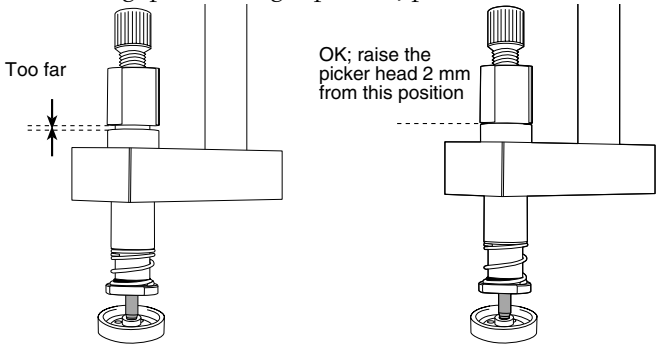
3 If it appears that the picker head will miss the centre of the rinse station, adjust the X/Y-position of the picker head as appropriate and continue to lower the picker head.



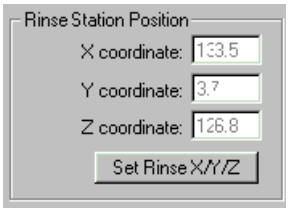
4 When the picker head is just above the rinse station, adjust the X/Y-position so that it is centrally placed.

5 Lower the picker head gently into the rinse station.

6 If a gap is formed at the top of the picker head piston, then the picker head has been lowered too far. Raise the picker head slightly so that the gap is no longer present, plus an additional 2 mm.



7 Press the **Set Rinse X/Y/Z** button.



3.5.4 Setting the positions of the microplates

The microplates are numbered as illustrated in the **System Setup** screen. They should be placed with the A1 well facing the operator. During System Setup only this well has to be defined for each plate.

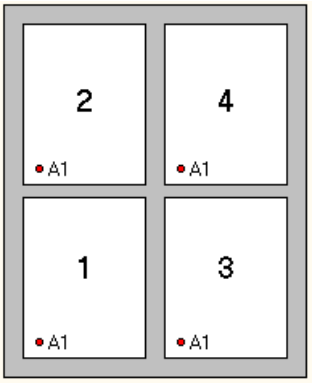
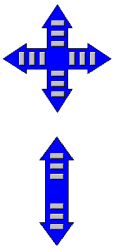


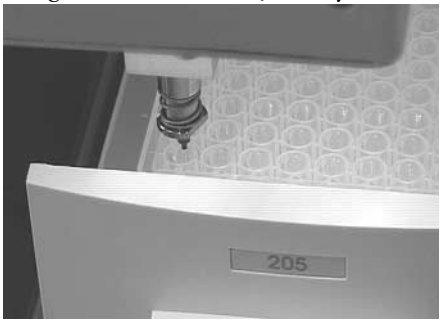
Fig 3-11. The numbering of the microplates.

To set the positions of the microplates:

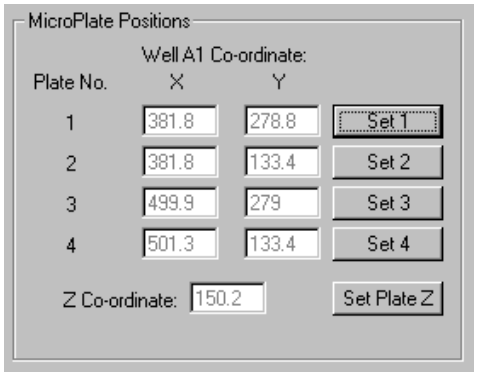


1 Using the **Move X/Y** arrow, move the picker head until it is centrally placed above the A1 well of microplate 1.

2 Using the **Move Z** arrow, slowly lower the picker head into well A1.



3 The current position of the picker head is continuously read back from the instrument and displayed in the corresponding text boxes:



4 If it appears that the picker head will miss the centre of the well, adjust the X/Y-position of the picker head as appropriate and continue to lower the picker head. When the picker head is in the A1 well, adjust it so that it is centrally placed.

5 The Z-position of the picker head should be adjusted so that approx. 2 mm of the tip is within the well. This will enhance the dispensing efficiency of the spot picker.

6 When the picker head is correctly positioned in the well, press the **Set 1** button.

7 At the first microplate, press the **Set Plate Z** button—this sets the Z-height for all of the microplates and does not need to be set for each plate individually.

CAUTION! Raise the picker head before moving to the next A1 well.

8 Repeat steps 1-6 for the remaining microplates, without pressing the **Set Plate Z** button.

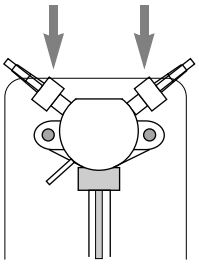
3.6 Setting picking parameters

Before picking spots from a gel, a number of **Picking Parameters** must be set, as well as **Gel Z Position** and **Marker Area.** Instructions for these settings can be found in the *Ettan Spot Picker User Manual*.

3.7 Priming the system

Priming the syringe removes any air bubbles in the spot picker tubing and also rinses the tubing with fresh solution. It is recommended that 5-10 priming strokes are used.

Note: It is important to ensure that the position of the rinse station is correctly set in the System Setup (see Section 3.5.3), before priming the system.

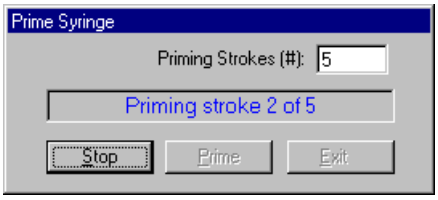


1 Check that the tube fittings at the valve are properly tightened.

2 Select **Tools:Prime Syringe**.

3 In the **Prime Syringe** window, enter the number of priming strokes and press the **Prime** button.

4 The **Stop** button is now enabled and can be used to interrupt the priming.



3.8 Calibrating the scanner

3.8.1 Background

Ettan Spot Picker uses the X/Y-image co-ordinates of protein spots to determine the location of the gel plugs to be removed. Some imaging systems have inherent imperfections, which lead to a slightly distorted gel image. The distortion of the image will lead to a distortion of the spot positions. The picker head will move to a position which, although the correct location that it has been sent to, is not representative of the true location of the protein spot.

To correct for such distortions, the ImageScanner can be calibrated using a separate software that is included in the ImageScanner Geometry Calibration Kit. The ImageScanner Geometry Calibration Tool software corrects for the distortions of the gel image that are introduced by the imaging equipment, by correcting the pixel co-ordinates of the spots contained within the pick list.

3.8.2 Calibration procedure

The calibration works in the following way:

- A special calibration sheet is imaged on the scanner
- The location of the spots in the subsequent image of the calibration sheet is determined
- The image distortion is calculated using the differences between the known co-ordinates of the calibration sheet and the measured spot locations
- The image distortion data is stored as a correction table for the scanner
- A gel pick list that is loaded into the Ettan Spot Picker Instrument Control Software is modified using the correction table for the particular scanner that was used when scanning the gel

For detailed instructions, see the documentation on the CD-ROM included in the ImageScanner Geometry Calibration Kit.

4 Maintenance

4.1 Introduction

Regular maintenance is important for safe and trouble-free operation of the Ettan Spot Picker. The user should keep the instrument clean and calibrate the camera whenever a picker head has been replaced. Preventive maintenance should be performed on a yearly basis by qualified service personnel.

This chapter provides instructions for user maintenance and service operations, and a schedule for preventive maintenance.

Contact your Amersham Biosciences representative for more service information.

WARNING! Never put your hands underneath the picker head during instrument operation. The picker head has the capacity to puncture skin. In the event of an emergency, press the **Stop** button on the front panel.

WARNING! MOVING PARTS. The picker head and camera assembly can make sudden, rapid movements. Always switch off mains power before replacing parts or cleaning the instrument.

4.2 Cleaning procedures

4.2.1 Cleaning the instrument regularly

For proper function, the Ettan Spot Picker should be kept clean and dry. Chemical stains and dust should be removed.

1 Remove the gel tray and the microplate racks from the locator plate.

2 Wipe the instrument cover with a soft damp tissue. If needed, use a mild detergent to remove stains.

3 Clean the gel tray and the microplate racks using a mild detergent.

4 Let the instrument dry completely before use.

4.2.2 Cleaning the instrument after spot picking

When a picking run is completed:

1 Remove the gel from the gel tray and store/discard as appropriate.

2 If the gel is to be discarded, scrape the gel off the glass plate with a plastic spacer or similar object. Then put the plates into a 5% Decon solution overnight to remove any gel fragments which adhere to the plate.

3 Rinse the buffer lines with distilled water using the prime syringe tool.

4 Rinse the liquid out of the gel tray with distilled water and leave the gel tray to dry.

4.3 Changing picker head

The Ettan Spot Picker can be used with a range of picker heads, with varying diameter and height. The picker heads are easily changed by a simple screw-in/screw-out method. After changing a picker head, a camera calibration should be performed, to establish the offset between the picker head and the camera lens. The gel Z-position may also have to be adjusted in the System Setup.

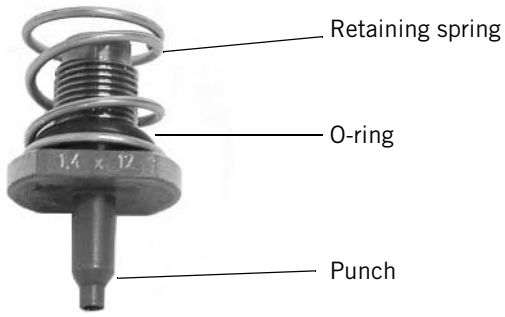
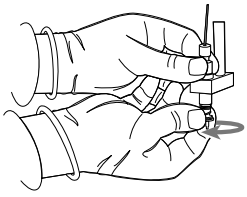


Fig 4-1. The picker head.

4.3.1 Removing the picker head

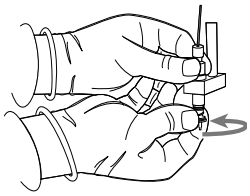
WARNING! Switch off mains power to the Spot Picker before removing the picker head.



1 When mains power has been switched off, move the picker head to a convenient position to work in.

2 Hold the top of the picking piston with one hand, and with the other unscrew the picker head from the base of the piston.

3 When the picker head is completely unscrewed from the base of the piston, place it in a safe location until it is required once more. Make sure that the retaining spring is also kept safe.



4.3.2 Attaching a new picker head

1 Place the retaining spring over the screw thread on the top of the picker head.

2 Screw the picker head onto the base of the piston, making sure that the screw thread is inside the piston. At the same time, hold the top of the piston steady with your other hand.

3 Slowly screw the picker head into the piston, while holding the top of the piston still with the other hand. The picker head only needs to be tightened to finger tight.

Fig 4-2. Final appearance of the assembled picker head.

Proceed by calibrating the distance between the picker head and the camera (see Section 4.4).

4.4 Calibrating the camera

4.4.1 Required accessories

You need the following accessories for the camera calibration:

- White sheet for calibration, code no. 18-1143-51
- Camera calibration foot, code no. 18-1143-33
- Double-sided adhesive tape

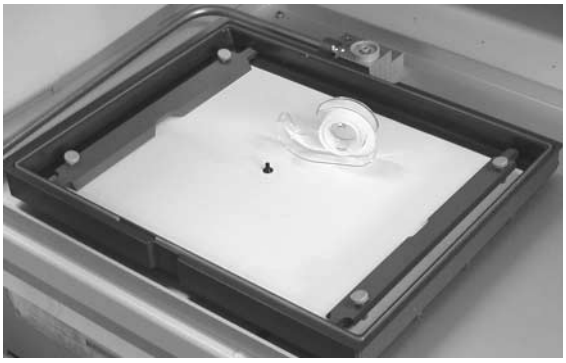


Fig 4-3. Accessories for camera calibration.

4.4.2 Focusing the lens

Before starting the camera calibration, check that the camera lens is focused on the bottom of the tray:

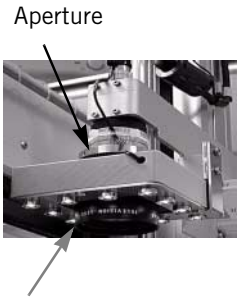
1 Use the Click and Pick screen for the adjustments: select **Tools:Click 'n' Pick.**

2 Place the white calibration sheet in the tray.

3 Place an object on the sheet, e.g. a piece of paper with printed text.

4 With the **Set Contrast** and **Set Brightness** controls in the centre positions, adjust the aperture to normal brigthness.

5 Adjust the focus ring on the lens to a sharp picture.

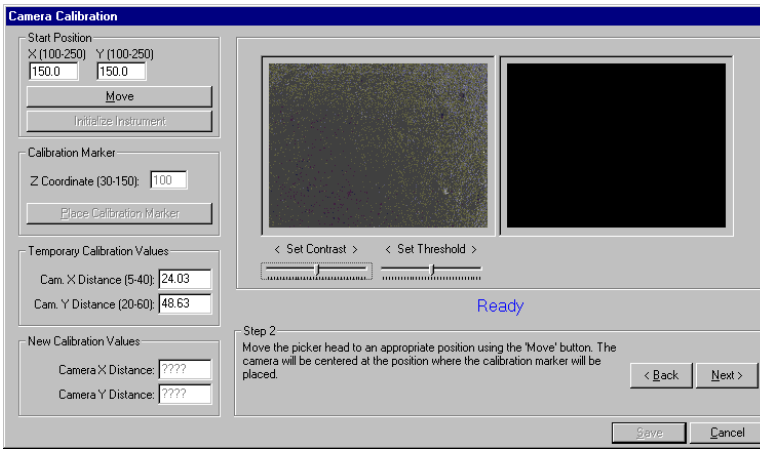


4.4.3 Calibration procedure

CAUTION! Before starting camera calibration, make sure that the Z-arm is properly mounted, as high as possible on the Y-arm.

1 In the main application window, under **Load Pick List,** select marker type **Black Markers.**

2 Select **System:Camera Calibration.**



3 Click on **Move** to move the camera to the position where the calibration foot will be placed (X=150, Y=150).

4 Place the calibration foot onto the picker head.

5 Locate the calibration position by placing an object under the camera or drawing a line with a pencil. View the left window of the **Camera Calibration** screen for orientation.

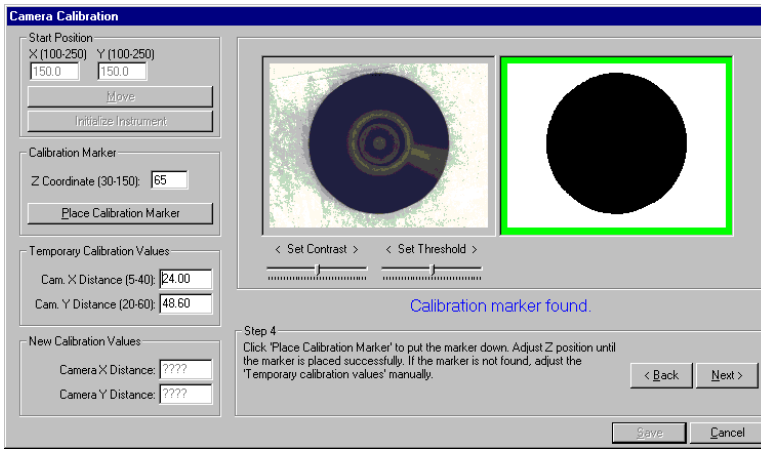
6 Place a strip of double-sided adhesive tape across the calibration position.

7 Click **Place Calibration Marker:** the picker head moves to the calibration position, and down towards the tray. The picker head stops at the Z-coordinate shown under **Calibration Marker**—the default Z-height is 100, which will be above the tray. The picker head immediately returns, and the video camera is moved, attempting to locate the calibration foot.

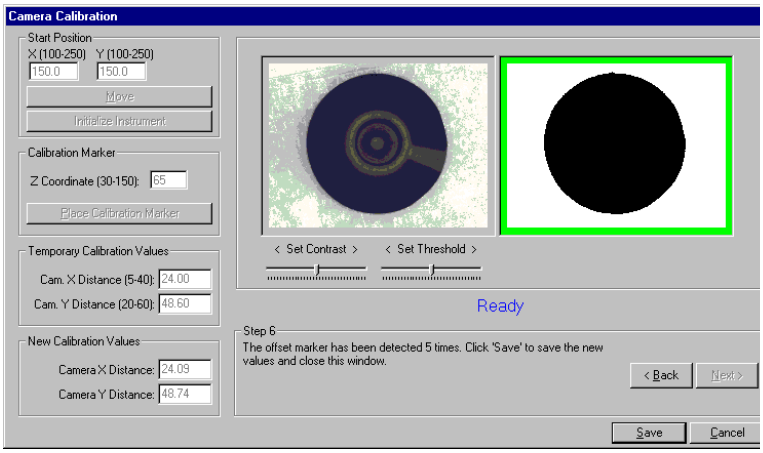
8 Change the **Z Coordinate** stepwise to lower values and click on **Place Calibration Marker** again. Repeat until the calibration foot is successfully attached onto the tape. The final Z-coordinate is normally 65-70.

Note: *Check before each step that the remaining distance is not smaller than the change of Z-coordinate.*

9 When the calibration foot has been placed onto the tape, and then identified by the camera, the frame around the detection window changes from red to green.



10 Repeat the procedure four times. When the calibration has been successfully performed, click on **Save** to save the new calibration values.



Troubleshooting the camera calibration procedure

- If the calibration foot is not visible in the camera view window although it is underneath the camera, adjust the aperture until the calibration foot is visible
- If the calibration foot is visible in the camera view window but not visible in the detection window, adjust the **Contrast** and **Threshold** sliding bars until an image is visible in the detection window
- If the calibration foot is not placed on the tape, decrease the Z-coordinate to make the picker head move closer to the tray

4.5 Replacing the mains fuse

A blown fuse may indicate the existence of another problem in the instrument. If the replacement fuse blows, don't try others. Contact your local Amersham Biosciences representative.

WARNING! For continued protection against risk of fire, replace with fuses of the same type and rating only.

To replace the mains fuse:

1 Disconnect the mains power cord from the power outlet and from the rear panel socket.

2 Locate the fuse drawer on the rear panel.

3 Insert a small screwdriver into the notch next to the fuse drawer.

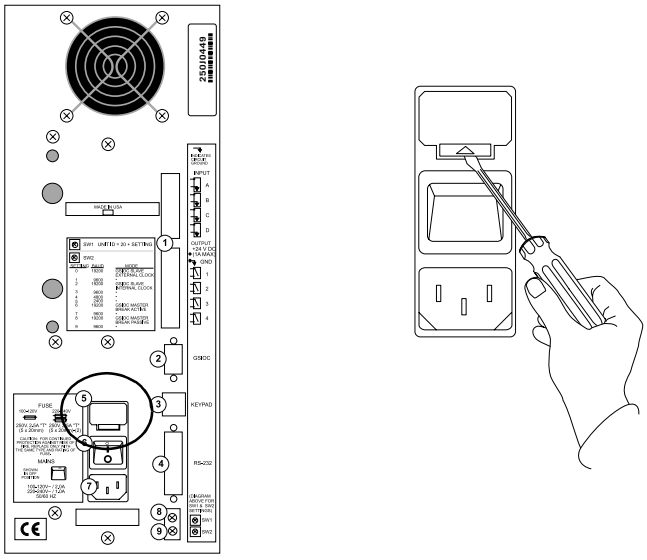
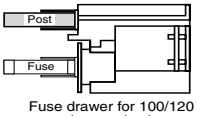
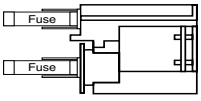


Fig 4-4. Replacing the mains fuse.



Fuse drawer for 100/120 voltage selection



Fuse drawer for 220/240 voltage selection

4 Twist the screwdriver to open and remove the fuse drawer. The fuse drawer contains one 2.5 A “T “ slow-blow fuse (5 x 20 mm size) for a 100/120 voltage selection. It contains two 2.5 A “T “ fuses for a 220/240 voltage selection.

5 Remove the old fuse(s) and insert the new fuse(s).

6 Insert the fuse drawer into its receptacle on the rear panel.

4.6 Preventive maintenance

Table 4-5 lists the preventive maintenance (PM) operations that should be performed by qualified service personnel during the yearly service visit.

Component/function	*Maintenance action*
Ettan Spot Picker system	Overall inspection and cleaning
Connections	Check that all cables are properly connected and undamaged
X-arm shaft	Lubricate
Z-arm shaft	Clean
Piston seal	Repair if worn or damaged
Homing phase	Check function
X/Y positions	Check function and adjust if necessary
Camera-picker head distance	Perform camera calibration
System set-up positions	Inspect and adjust if necessary
Picking sequence	Check function
Log file	Check for errors that may require action

Table 4-5. Preventive maintenance schedule.

5 Troubleshooting

This chapter provides basic diagnostic and troubleshooting guides. The diagnostic guide is based on the error messages that are displayed by the Ettan Spot Picker Instrument Control Software (see Table 5-1). The basic troubleshooting guide focuses on error symptoms related to instrument operation (see Table 5-2).

For errors related to gel picking, refer to the Troubleshooting chapter in the *Ettan Spot Picker User Manual*.

Fig. 5-1 shows a typical error message. A short explanation is displayed by clicking on **Details>>>.**

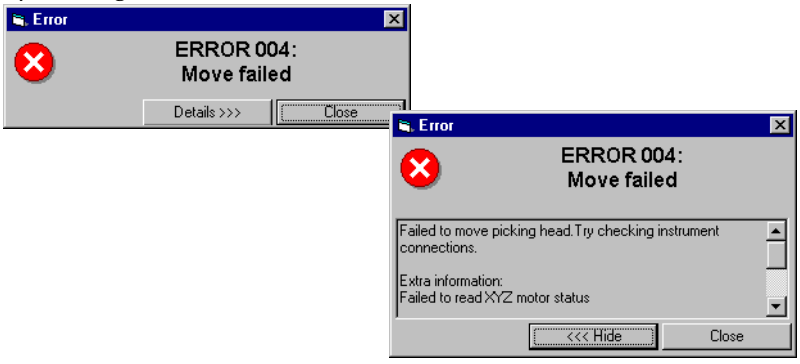


Fig 5-1. Typical error message.

5.1 Diagnostics

Error message	*Details/Possible cause*	*Corrective action*
001:Reset failed	Failed to reset the instrument.	Try checking instrument connections.
002:Homing failed	Failed to home the picker head.	Try checking instrument connections.
003:Homing failed	Failed to home dilutor.	Try checking instrument connections.
004:Move failed	Failed to move the picker head	Try checking instrument connections.
005:Move timed out	Failed to move the picker head.	Try checking instrument connections.
006:Movement out of range	Movement outside travel range.	When picking: Try changing system setup.
007:Dilutor operation timed out	Dilutor operation failed.	Try checking instrument connections.
008:Dilutor operation failed	Dilutor operation failed.	Try checking instrument connections.
009:No connection	Failed to read the picker head position from the instrument.	Try checking instrument connections and/or reinitialize the instrument.
010:No connection	Failed to read syringe content from the instrument.	Try checking instrument connections and/or reinitialize the instrument.
011:Read version failed	Failed to read instrument version.	Try checking instrument connections and/or reinitialize the instrument.
012:Read version max flow failed	Failed to read dilutor max flow.	Try checking instrument connections and/or reinitialize the instrument.
013:Read travel range failed	Failed to read the picker head travel range.	Try checking instrument connections and/or reinitialize the instrument.
014:Not used		
015:File error	Could not access file, or missing file	

Error message	*Details/Possible cause*	*Corrective action*
016:Not used		
017:Not used		
018:File error	Trying to access output directory, which is not valid or write-protected	Enter a valid output directory, or change attribute for directory, e.g. uncheck “read-only”
019:File error	Could not access file, or missing file	Example: move scanner calibration file to correct directory
020:File format error	Cannot recognize the file format	Errors in pick list, e.g. multiple IR1 or IR2 coordinates.
021:Internal error	An application error has occured	If the problem persists, please contact Amersham Biosciences support
022:Scanner correction failed	Failed to calculate scanner correction	Try running the scanner calibration program again and replace the calibration file
023:Homing failed (X axis)	Homing failed for the X-axis. The cause may be that the instrument has collided while moving in the X-direction (left/right)	Run the 'Set Model 215' program and use the 'Home Phase' Tool.For details, see the user manual
024:Homing failed (Y axis)	Homing failed for the Y-axis. The cause may be that the instrument has collided while moving in the Y-direction (forward/backward)	Run the 'Set Model 215' program and use the 'Home Phase' Tool.For details, see the user manual
025:Homing failed (X and Y axes)	Homing failed for the X and Y-axes. The cause may be that the instrument has collided while moving diagonally	Run the 'Set Model 215' program and use the 'Home Phase' Tool.For details, see the user manual

Table 5-1. Diagnostic guide.

5.2 Troubleshooting

Error symptom	*Possible cause*	*Corrective action*
No picture from the camera	Camera not connected to the PC	Check camera cable and reconnect
Reference detection unstable	Contrast not properly set	Adjust the aperture setting on the camera
Light not coming on.	Power supply from 24 V output disconnected	Check power cable from light to 24 V power supply
	Internal fuse has blow	Call your Amersham Biosciences service representative
No movement from the Spot Picker following command sent from the PC	Instrument not plugged in or mains switch turned off	Check that the Spot Picker is connected to a wall socket and that the mains switch is in the ON (**1**) position
	The **Stop** button has been pressed	Press the **Start** button on the Spot Picker
	RS232 cable not properly connected	Check RS232 cable and ensure that it is plugged into the correct port
Other problems		Call your Amersham Biosciences service representative

Table 5-2. Troubleshooting guide.

6 Moving and reinstalling the system

TheEttan Spot Picker system is installed and tested by Amersham Biosciences service personnel after delivery. This chapter provides instructions for reinstallation, should you need to move the instrument to another location.

WARNING! Always shut down Ettan Spot Picker and the PC before disconnecting or connecting cables. See instructions in Chapter 4.

Before moving the system, check that the new location fulfils the site requirements listed below.

6.1 Site requirements

6.1.1 Bench space

The Spot Picker should be placed on a stable and level laboratory bench. Allow space for a water bottle on the left hand side of the instrument, and for the computer and monitor on the right hand side.

Note that the Z-arm can move up to 56 cm above the bench. Make sure that any shelves are mounted at sufficient height.

Dimensions, Ettan Spot Picker instrument	91 x 61 x 56 cm (W x D x H)

6.1.2 Environment

Temperature, operating	18-30 °C (65-86 °F)
Maximum relative humidity	80% non-condensing
Air pressure	75 - 105 kPa (up to 2000 m altitude)

The system must not be exposed to direct sunlight. Dust in the atmosphere should be kept to a minimum.

6.1.3 Power requirements

Mains voltage	100-120 V~ or 220-240 V~ ±10%, 50/60Hz, single phase
Power consumption	200 VA

6.1.4 Media supply

The Ettan Spot Picker requires a supply of deionized or distilled water (Milli-Q), or the used wash/destain solution.

6.2 Moving the instrument

WARNING! The Ettan Spot Picker instrument weighs about 41 kg. Two persons are required to lift the instrument.

CAUTION! Do not use the Y-arm as a handle when moving the Spot Picker. Always lift the instrument from the base.

To move the system to another room:

1 Shut down the Ettan Spot Picker and the PC.

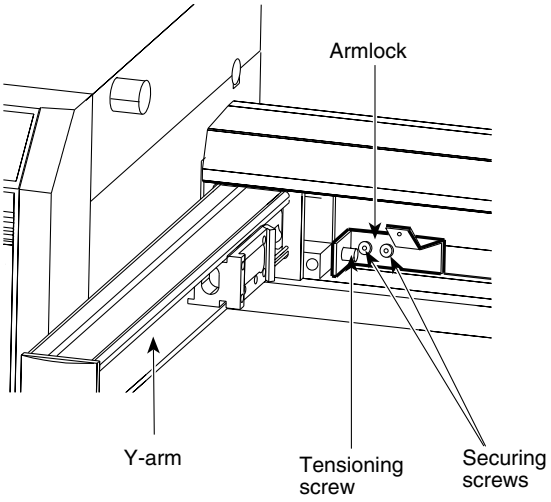
2 Disconnect the communication cables and the frame grabber cable at the rear panels on the instrument and the PC.

3 Disconnect both mains supply cords from the wall outlets, the instrument and the PC.

4 Disconnect the monitor cable and the monitor mains supply cord at the PC and the monitor.

5 To prevent mechanical damage, install the armlock securing the Y-arm:

- mount the tensioning screw that immobilizes the Y-arm
- mount the two securing screws that hold the armlock in place



6 Carefully place the instrument, the PC and the monitor on a trolley.

CAUTION! Lift the instrument at the base. Do not use the X- or Y-arms as handles.

7 Move the system to the new location.

6.3 Reinstalling the Ettan Spot Picker

1 Select a place for the Spot Picker where it is possible to access the mains power switch on the rear panel of the instrument.

2 Remove the armlock.

3 If the Z-arm has been removed, reinstall in its highest position.

If you need to reinstall the Ettan Spot Picker Instrument Control Software, see instructions in Section 6.4.

6.3.1 Electrical connections

Reconnect the cables according to the diagram in Fig. 6-1.

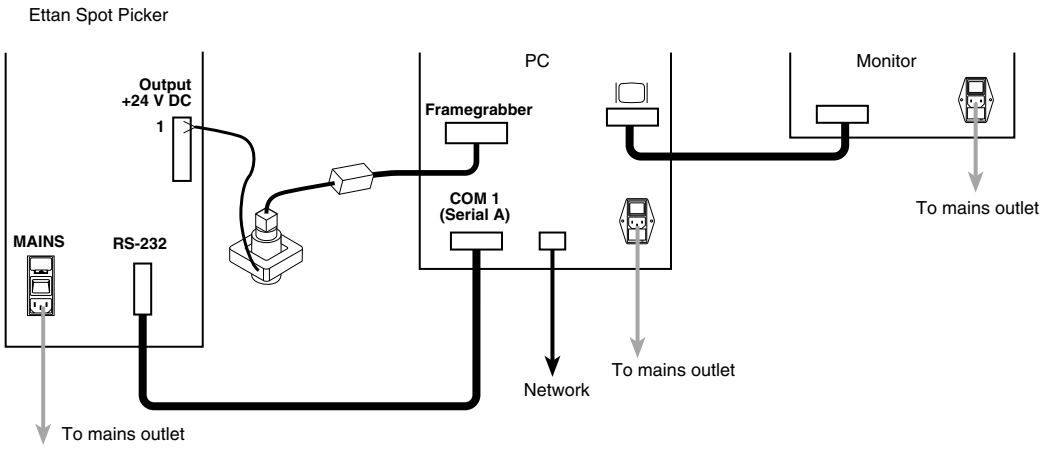


Fig 6-1. Electrical connectors.

WARNING! Use only the power cords and cables supplied with the Ettan Spot Picker system. Use of incorrect power cords can cause damage to the instrument. Use of damaged power cords can cause injury.

Connecting to mains power

WARNING! If an Ettan Spot Picker configured for 100-120 V is connected to 220-240 V, the instrument can be severely damaged. Make sure that the correct fuse drawer for the voltage range is installed.

During the original installation, the Ettan Spot Picker is configured for one voltage range by installing the appropriate fuse drawer. Before reinstalling the instrument, make sure that the correct fuse drawer is installed for the mains voltage at the new location. If you need to change the mains voltage configuration, refer to instructions in Section 4.5 for installing mains fuses.

To connect the Ettan Spot Picker system to mains power:

1 Connect one end of the supplied mains cable to the mains inlet on the Spot Picker and the other end to a mains supply outlet with protective ground.

2 Check that the mains voltage marking on the PC corresponds to the mains supply voltage.

3 Connect one end of the supplied mains cable to the mains inlet on the PC and the other end to a mains supply outlet with protective ground.

4 Connect the supplied monitor power cable between the mains inlet on the monitor and a mains supply outlet with protective ground.

Connecting to the PC

Connect the supplied communication cable between the **RS-232** connector on the Ettan Spot Picker and the **COM1** port on the PC. Check that the switches **SW1** and **SW2** are set as shown in Fig. 6-2.

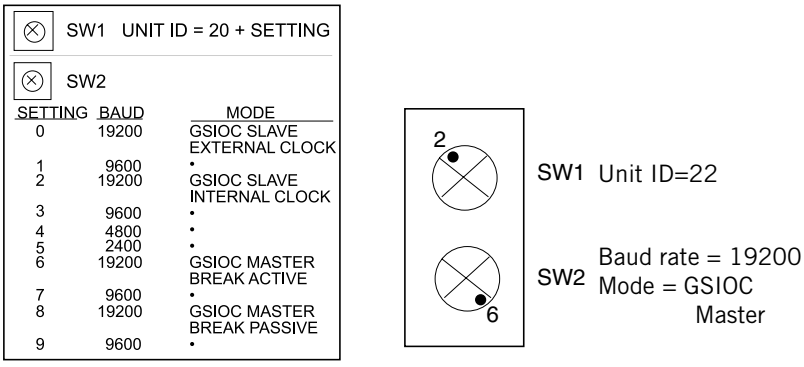


Fig 6-2. Communications settings on the Ettan Spot Picker.

6.3.2 Power-up check

To verify the function of the Spot Picker at the new location:

1 Switch on the mains power at the rear of the instrument.

2 Check that the **Power** indicator lights.

3 Switch on the mains power to the PC.

4 To start the Ettan Spot Picker Instrument Control Software, Select **Programs:Ettan Spot Picker:Ettan Spot Picker** from the Windows **Start** menu:

5 Click on **OK** in the **Homing** screen to start the homing procedure, where the picker head moves to the home position.

6.3.3 Camera calibration

Perform a camera calibration, see Section 4.4.

6.3.4 Shutdown

To shut the Ettan Spot Picker instrument down:

1 Close down the Ettan Spot Picker software by choosing **File:Exit**.

2 Switch off mains power on the Ettan Spot Picker rear panel.

6.4 Reinstalling the Ettan Spot Picker Instrument Control Software.

IMPORTANT! Before installing the software, back up your result files.

To reinstall the control software, run the installation program **setup.exe** from the Ettan Spot Picker CD-ROM and follow the instructions on the screen.

The installation program installs the software in the following order:

1 Instrument software.

2 Frame grabber software.

3 Ettan Spot Picker software.

The PC must be restarted after each step.

For information on the instrument software and the frame grabber software, read any "readme" files in the \picker and \framegrabber folders on the CD-ROM.

During the software installation, factory defaults are stored for system parameters. Therefore, you need to:

- re-calibrate the camera (Section 4.4)
- perform a system setup (Section 3.5)

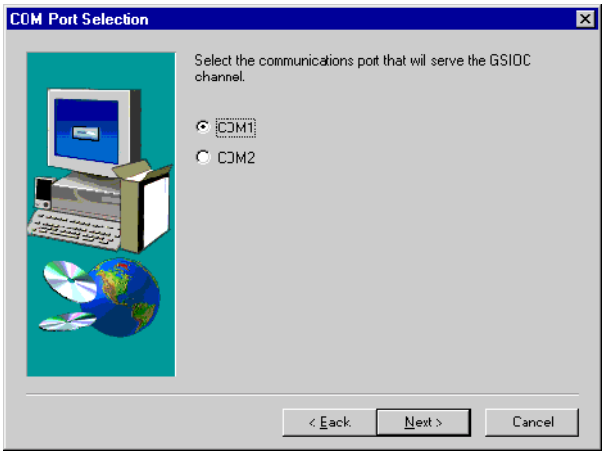
6.4.1 Instrument software

Before starting installation, make sure that the Windows control panel **Regional Settings** is set up as follows:

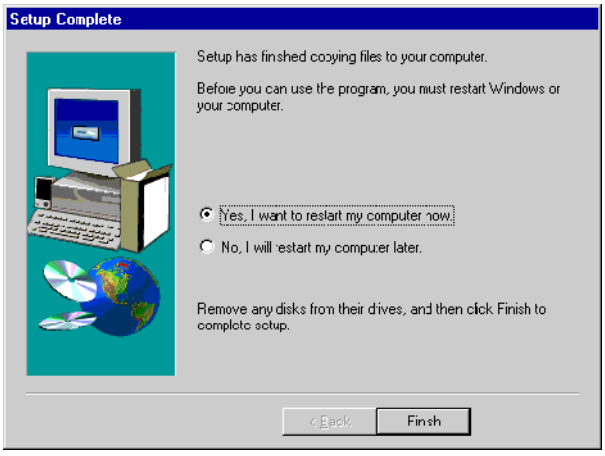
Regional Settings: English (United States).

Input Locales: choose your national keyboard.

1 Select the appropriate COM-port where the instrument communication cable is attached. Click on **Next**.

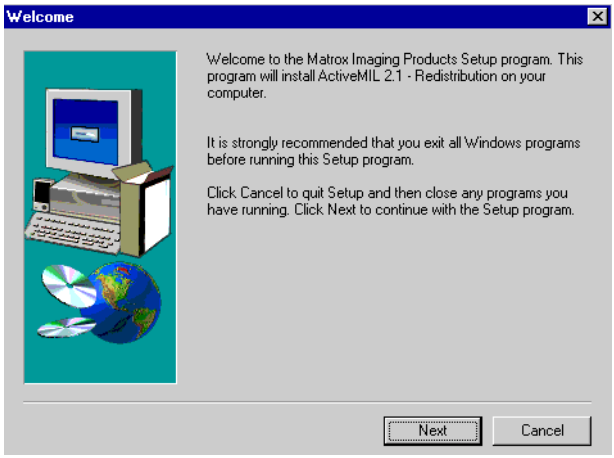


2 When the instrument software is installed, click on **Finish** to restart the PC.

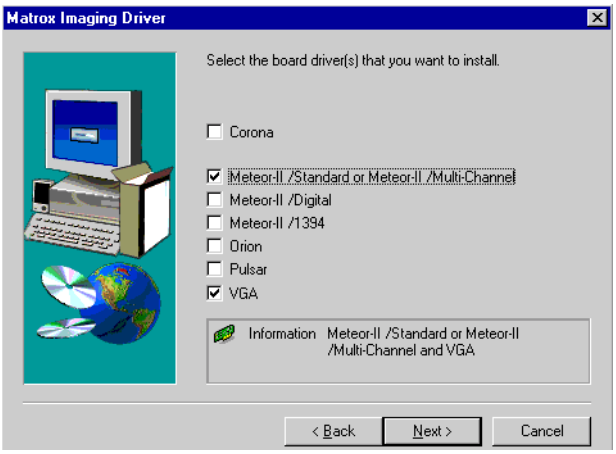

Setup Complete
Setup has finished copying files to your computer.
Before you can use the program, you must restart Windows or your computer.
Yes, I want to restart my computer now.
No, I will restart my computer later.
Remove any disks from their drives, and then click Finish to complete setup.
< Back
Finish

6.4.2 Frame grabber software

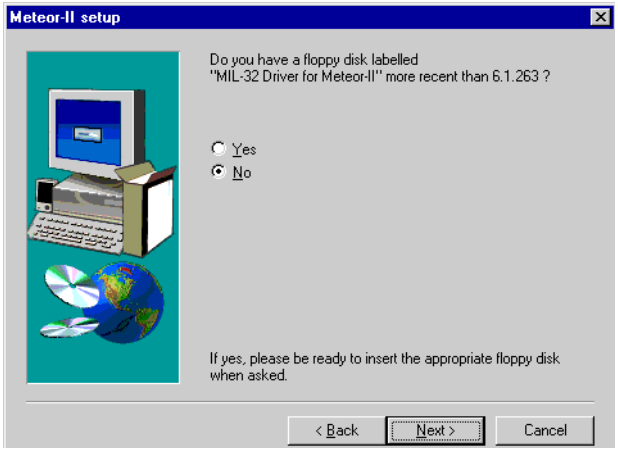
1 Click on **Next** in the first screen to start the installation.


Welcome
Welcome to the Matrox Imaging Products Setup program. This program will install ActiveMIL 2.1 - Redistribution on your computer.
It is strongly recommended that you exit all Windows programs before running this Setup program.
Click Cancel to quit Setup and then close any programs you have running. Click Next to continue with the Setup program.
Next
Cancel

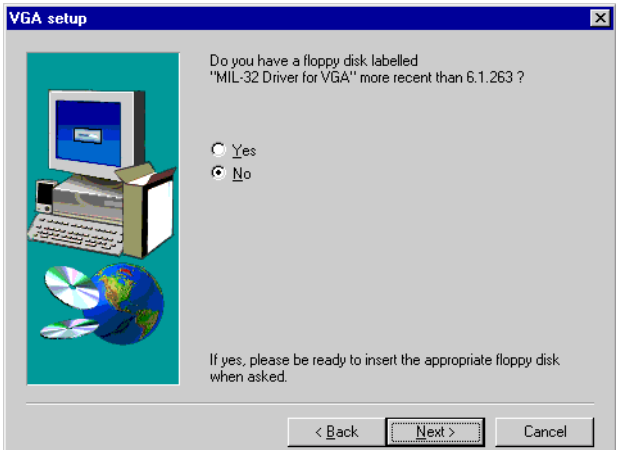
2 Select **Meteor-II** and **VGA** drivers. Click on **Next.**



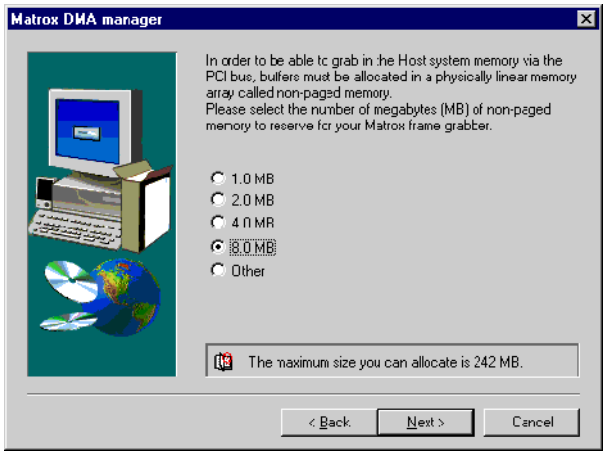
3 Select **No** for new MIL-32 driver for Meteor-II. Click on **Next.**


Meteor-II setup
Do you have a floppy disk labelled
"MIL-32 Driver for Meteor-II" more recent than 6.1.263 ?
Yes
No
If yes, please be ready to insert the appropriate floppy disk
when asked.
< Back
Next >
Cancel

4 Select **No** for new MIL-32 driver for VGA. Click on **Next.**


VGA setup
Do you have a floppy disk labelled
"MIL-32 Driver for VGA" more recent than 6.1.263 ?
Yes
No
If yes, please be ready to insert the appropriate floppy disk
when asked.
< Back
Next >
Cancel

5 Select **8.0 MB** memory buffer size. Click on **Next**.


Matrox DMA manager
In order to be able to grab in the Host system memory via the PCI bus, buffers must be allocated in a physically linear memory array called non-paged memory.
Please select the number of megabytes (MB) of non-paged memory to reserve for your Matrox frame grabber.
1.0 MB
2.0 MB
4.0 MB
8.0 MB
Other
The maximum size you can allocate is 242 MB.
< Back
Next >
Cancel

6 Click **OK** to start copying files.

Matrox Imaging Products redistribution
Setup is now ready to copy files to your system.
If you want to change any settings, click Back.
Click Ok to begin copying files.
< Back
Ok

7 When installation of the frame grabber software is finished, select **Yes** and click on **Finish** to restart the PC.

Matrox Imaging Products - Redistribution setup
Setup has successfully installed the Matrox Imaging Product to your computer.Before you can use it, you must restart your computer.
Yes, I want to restart my computer now.
No, I will restart my computer later.
When your computer has restarted, you can use your applicationwith the Matrox Imaging Product.
< Back
Finish

6.4.3 Ettan Spot Picker Software

The installation procedure for the Ettan Spot Picker software is straight-forward and requires no special selections. For easy set-up, accept the suggested file location and program group.

Close any running application, then click **OK** in the first screen to start the installation (see Fig. 6-3).

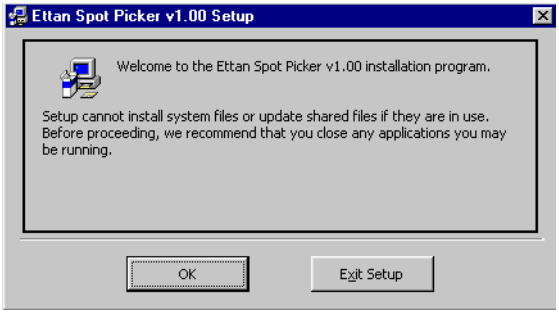

Ettan Spot Picker v1.00 Setup
Welcome to the Ettan Spot Picker v1.00 installation program.
Setup cannot install system files or update shared files if they are in use. Before proceeding, we recommend that you close any applications you may be running.
OK
Exit Setup

Fig 6-3. Ettan Spot Picker software installation, start screen.

7 Reference information

7.1 The Spot Picker Instrument

The Ettan Spot Picker is a robotic system that can move the picker head in three directions: X, Y and Z. The syringe pump allows precise handling of liquid and picked gel plugs. The valve next to the syringe pump controls the flow from the liquid supply, and to/from the picker head.

The picker head is rinsed between each pick, and waste liquid is dispensed into the rinse station. The waste liquid flows through the waste tube to a container placed below the Spot Picker.

The picker head movements are guided by a camera system. The camera that is attached to the Z-arm is connected to the PC via a frame grabber card. Reference markers on the gel are identified using the camera. This enables the pick list co-ordinates from the scanned gel to be transferred to picker head positions during automated picking. The operator can also pick gel plugs manually using the camera image for orientation.

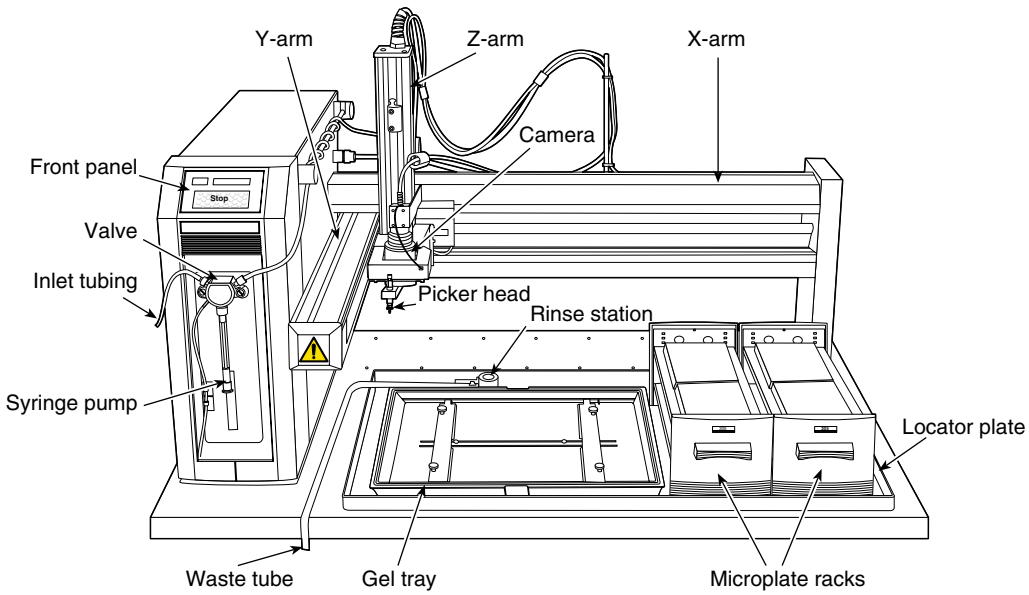


Fig 7-1. The Ettan Spot Picker.

A gel tray is placed on the locator plate on the base of the instrument. The gel to be picked is fastened in the tray using gel holders. When calibrating the camera, a white sheet is fastened in the tray. The white sheet is also used during manual picking, to enhance contrast in the camera image.

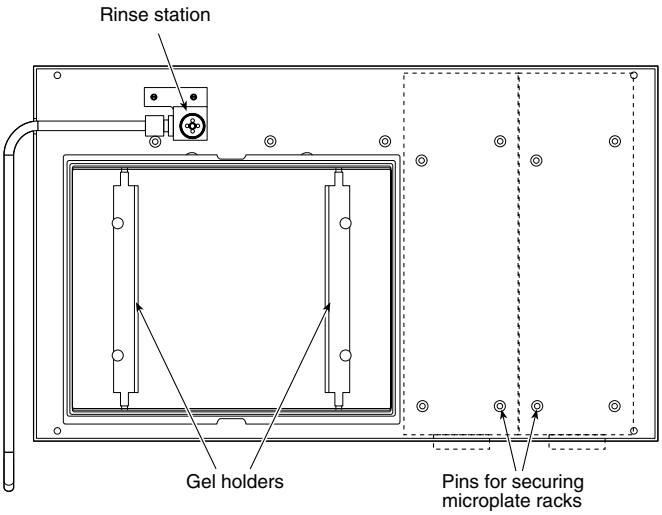


Fig 7-2. Locator plate with gel tray.

Two racks for microplates are placed beside the gel tray. Up to four microplates can be placed in the racks at the same time. However, the software allows the operator to change microplates during picking, enabling a large number of gel plugs to picked in one run.

7.1.1 Front panel

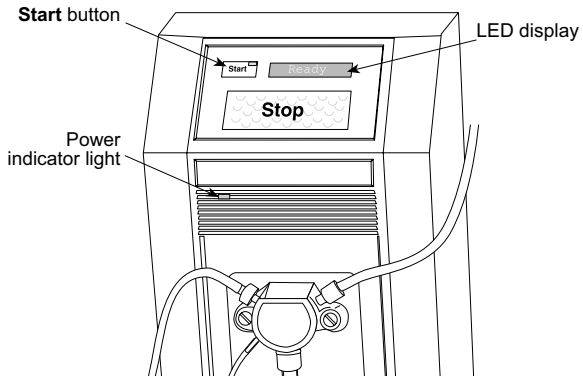


Fig 7-3. Indicators and controls on the front panel.

Indicator	*Colour*	*Function*
Power (unmarked)	green	Lights when mains power is connected to the instrument and the mains switch on the rear panel is on (in the **1** position).
Start	yellow	Lights when the **Start** button is pressed to home the picker head.
LED display	yellow text	Shows the current status of the Spot Picker and any error codes as they are encountered (see Chapter 5).

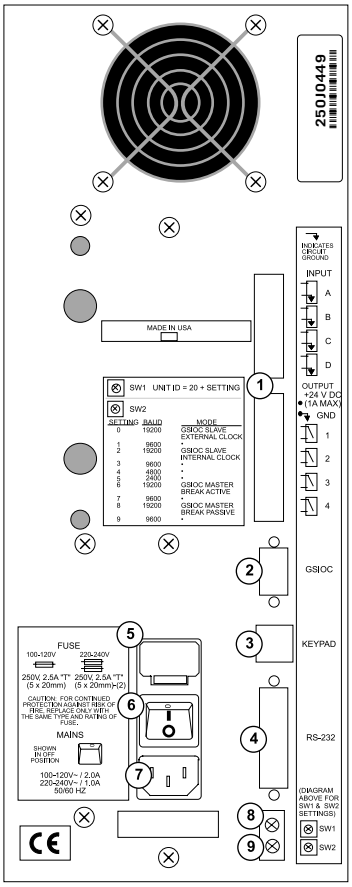
Table 7-4. Indicators on the front panel.

Button	*Function*
Start	When the **Start** button is pressed, the picker head moves to home position. The yellow LED lights as long as the button is pressed. The **Start** button can be used to home the picker head when the Ettan Spot Picker is first powered up or when the motors for the picker head have been relaxed.
Stop	When this button is touched, the instrument immediately ceases movement and all motors are de-energized. When the **Stop** button is pressed, the yellow LED in the **Start** button is turned off. Note that the **Stop** button is very sensitive, do not touch it unnecessarily during a run.

Table 7-5. Buttons on the front panel.

7.1.2 Connectors

All connectors are located on the rear panel of the instrument (see Fig. 7-6). The mains switch and mains fuse drawer are part of the mains inlet connector at the lower end of the panel.



Rear panel connectors and switches

1 LED driver output for camera
2 - 3 Not used
4 RS-232 port for connection to PC
5 Mains fuse drawer
6 Mains power switch
7 Mains power socket
8 Unit ID selector

Fig 7-6. Rear panel with mains power connector.

7.1.3 The picker head

The picker head is mounted on the Z-arm just behind the video camera. During a run, the picker head is lowered into the gel and on to the gel backing. The punch excises a gel plug by sideways movements ("jazz") and the gel plug is then aspirated into the picker head. The aspiration is achieved by the syringe pump via the liquid column in the tubing.

After moving to the microplate rack, the picker head dispenses the gel plug into a microplate well. Before the next pick, the picker head moves to the rinse station where it is rinsed by aspirating and dispensing liquid.

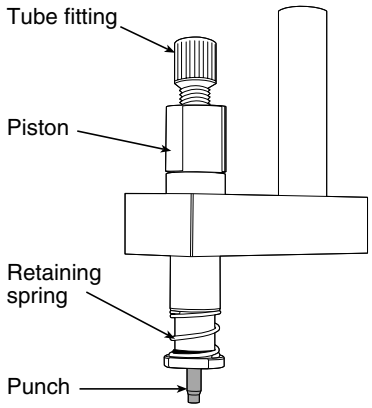


Fig 7-7. The picker head.

The retaining spring creates a pressure against the gel backing during picking, thus ensuring that the gel plug will be properly excised. When the Z-height is correctly adjusted, the picker head should be lowered until a gap is formed between the piston and the body of the picker head, thereby allowing the spring to press against the gel backing.

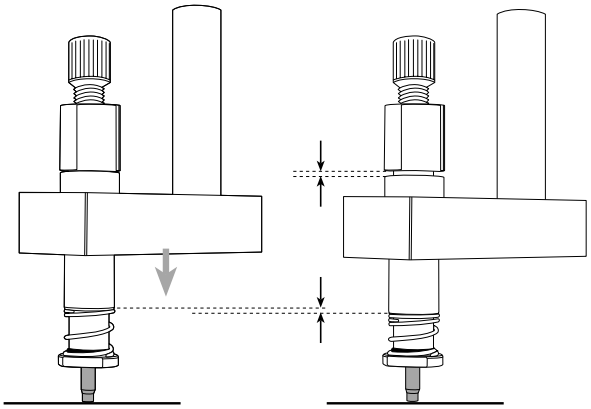


Fig 7-8. Spring action when lowering the picker head.

7.1.4 Video camera

The video camera is used to establish the coordinates for the gel to be picked. By detecting the reference markers on the gel and comparing with the coordinates in the pick list, the picking coordinates can be determined.

Since the camera lens is located at a distance from the picker head, its position relative to the picker head has to be precisely defined to ensure high precision picking. The camera calibration routine provides the means for this (see Section 4.4).

The operator needs to adjust focus and aperture for optimal imaging. Normally, an aperture of 8 is used. Brightness and contrast can be adjusted from the control software. The lamps surrounding the camera lens provide the required lighting for reference marker detection and camera calibration.

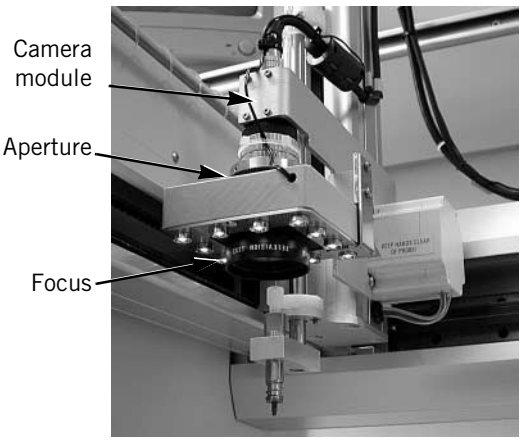


Fig 7-9. The video camera.

The video signal is processed by a frame grabber card in the PC before displaying the image in the Ettan Spot Picker Instrument Control Software.

7.2 Specifications

Basic specifications for the instrument can be found in the *Gilson 215 Liquid Handler User's Guide*. This section lists additional data for the Ettan Spot Picker.

7.2.1 Technical specification

Mains voltage	100-120 V~ ±10%, 50/60 Hz, or 220-240 V~ ±10%, 50 Hz, single phase (Installation category II)
Mains fuse	1× T2.5 A@110 V~, or 2× T2.5 A@220 V~
Power consumption	Max. 200 VA
Operating temperature	18-30 °C (65-86 °F)
Dimensions	91 × 61 × 56 cm (W × D × H)
Weight	41 kg
Sample source	Polyacrylamide gels cast on glass or GelBond PAG film backing
Maximum gel size	280 x 250 mm
Gel thickness compatibility	1, 1.5 mm
Picker head diameter	1.4, 2.0 mm
Detection system compatibility	Silver, coomassie, fluorescence

Table 7-10. Ettan Spot Picker technical specification.

7.2.2 Performance specification

Picking efficiency	>99% of spots transferred from gel to microplate
Picking capacity	9600 protein spots per pick list
Process capacity	96 samples in less than 30 minutes

Table 7-11. Ettan Spot Picker performance specification.

7.2.3 Regulatory requirements

The Ettan Spot Picker system meets or exceeds the standards listed in Table 7-12 .

Safety	UL 1260, UL 1492 (camera), CSA C22.2 - No. 151, IEC 1010-1
EMC	EN 50082-1, EN 50022 (frame grabber), FCC Class A
EMI	EN 50081-1, EN 50024 (frame grabber)

Table 7-12. Regulatory requirements, Spot Picker instrument.

7.3 Ordering information

Ettan Spot Picker, including control software, picker heads and reference markers, is ordered under Code No. 18-1145-28. The delivery package is detailed in Section 1.2.

7.3.1 Accessories

Table 7-13 lists the accessories that can be ordered from Amersham Biosciences.

Product	*Quantity*	*Code No.*
Picker head 1.4 mm for 1 mm thick gels	1	18-1147-03
Picker head 1.4 mm for 1.5 mm thick gels	1	18-1147-05
Picker head 2.0 mm for 1 mm thick gels	1	18-1143-64
Picker head 2.0 mm for 1.5 mm thick gels	1	18-1143-37
Reference markers, sheet incl. 560 pieces	2	18-1143-34
Gel holder	2	18-1143-50
Gel tray	1	18-1143-38
Glass lid for gel tray	1	18-1143-32
Syringe, 1 ml	1	18-1143-41
Waste bottle and tubing	1	18-1143-44
Tubing and fittings	1	18-1143-63
Inlet tubing package	1	18-1143-65
Rinse station	1	18-1143-31
Camera calibration foot	5	18-1143-33
White sheet for camera calibration and click-and-pick function	2	18-1143-51
Microplate rack	2	18-1143-43
RS 232 cable	1	18-1143-45

Table 7-13. Accessories for Ettan Spot Picker.

7.3.2 Related products

Table 7-14 list products that are related to Ettan Spot Picker, these products can be ordered from Amersham Biosciences.

Product	*Code Number*
Ettan DALT II large vertical gel system	*
Ettan DALT II Pre-cast Gel 12.5	17-6002-36
Ettan DALT II Buffer Kit	17-6002-50
Immobiline DryStrip gels and buffers	*
ImageMaster 2D Elite software	80-6350-56
ImageScanner	18-1134-45
ImageScanner Geometry Calibration Kit	18-1145-55
Typhoon 8600	*

Table 7-14. Products related to Ettan Spot Picker. For code numbers marked with an asterisk (*), see www.amershambiosciences.com

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